Graphene biosensor based on functionalized hydrophobins

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Abstract

Graphene field effect transistors (GFET) are sensitive to the variations in the charge density in the vicinity of the channel and because most biomolecules are charged, the detection is mostly label free. However, biorecognition can only be achieved by selective binding of the analyte, which requires functionalization of graphene surface with antibodies, DNA, peptides or proteins. As the defect free graphene surface is inert and the formation of covalent bonds hinders the electronic properties of graphene, many schemes for non-covalent binding have been developed, such as physisorption of aromatic molecules [1], thiol functionalization of nanoparticles [2] or peptide functionalization [3,4]. In covalent binding the sensing surface is usually graphene oxide (GO) or graphene damaged with oxygen or ammonia plasma treatments and electrical performance is sacrificed for increased binding. Our approach is based on the functionalization of the graphene by engineered hydrophobin proteins, which self-assemble on hydrophobic surfaces to an ordered monomolecular layer with known orientation.

Hydrophobins are protein amphiphiles having a hydrophobic patch in one end. Hydrophobins attach to hydrophobic substrates such as graphite or graphene [5]. Hydrophobins have been used to exfoliate thin graphene flakes from graphite and the binding of the protein on graphene was demonstrated with N-cysteine funtionalised hydrophobin (NCys-HFBI) layer on the graphene to which selective binding of mercaptosuccinic acid treated Au nanoparticles occurs [6].

Here we present a graphene FET biosensor with surface functionalization by tailored hydrophobic protein HFBI-ZE having a ZE-zipper amino acid chain and an analyte ZR-zipper amino acid (pI 11.7) which binds to the ZE (pI 4.1). The graphene sensor was fabricated on a highly doped (p-type) Si wafer with 300 nm SiO_2 on top by transferring CVD grown graphene to the SiO_2 surface. Graphene was then patterned using optical lithography and O_2-plasma. Graphene contacts were fabricated using lift-off and evaporation of Ti and Au with a thickness of 5 nm and 50 nm respectively. Protective ALD Al_2O_3 was deposited on the chip and holes were etched on to the contact pads and graphene channel. Pt was deposited on liquid electrode pads using lift off and evaporation. The chip was wire bonded to a chip carrier that was attached to a circuit board having electrical connections and fluidistic cell support mechanism. The fluidistic cell was fabricated from PDMS (SYLGARD 184) by using a mold followed by attachment of flexible tubes for fluid transport. No adhesion promotion such as O_2-plasma or corona discharge was used on the PDMS and the sensor chip because graphene is etched by the plasma. Instead, mechanical clamping was used to attach the PDMS fluidistic cell to the sensor. A computer controlled syringe pump was used to feed the protein and buffer solutions into the system. Figure 1A shows the schematic of the measurement setup. In the experiments the liquid gate potential (V_{gate}) was sweeped and the liquid potential (V_L) was measured using high ohmic voltage preamplifier.

Electrical characterization of the sensor was conducted in sodium phosphate buffer solutions having concentration of 0.1 M and a pH of 7. First the response of the clean sensor was measured in buffer. Next, a solution containing 100 µg/ml of HFBI-ZE protein was introduced to the system and was allowed to form a monolayer on the graphene. After flushing with buffer solution the response was recorded. Last, a solution containing 10 µg/ml of ZR-protein was introduced to the system to see the effects of binding. Figure 1B shows the measured graphene resistance plotted against the measured potential of the electrolyte V_L. A clear shift in the resistance vs. V_L curve is observed after the graphene surface has been covered with HFBI-ZE. After applying the ZR zipper protein analyte the curve shifts again producing a clear bio-response (see Fig. 1B).

Shifting of the resistance curve due to different protein coatings can be explained by gating effect caused by the charges in the proteins. HFBI-ZE has a negative charge at pH 7 moving the resistance curve and the Dirac peak to the right in Fig. 1B. The binding of the protein can also cause shifts in the curves due to interactions at the graphene interface. ZE-protein, on the other hand, is positively charged at pH 7. Therefore, binding of ZE should move the resistance curve to the left on V_L axis, which is precisely the case Fig. 1B.

To summarize, we have fabricated a graphene bio-sensor and a fluidic setup and measured the effect of graphene surface functionalization with HFBI-ZE protein and demonstrated the selective binding of the analyte ZR protein. Effect of the charged proteins and the binding of analyte molecule can be seen in the shift of the resistance curve and Dirac peak measured against the reference liquid electrode and can be explained by the gating induced due to charged molecules.

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Figures

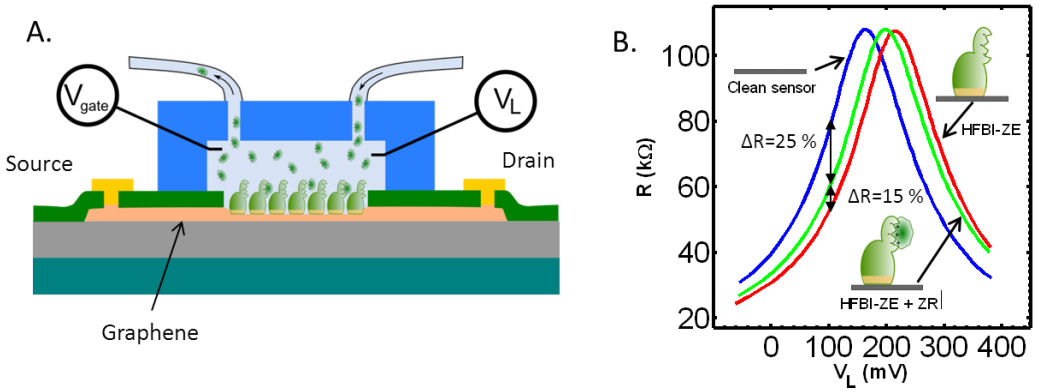

Figure 1 A) GFET biosensor setup and B) measured resistance (*R*) as a function of liquid potential (V_L). Clean graphene (blue) functionalized with HFBI-ZE protein before (red) and after (green) ZR zipper binding, which induces a 15 % shift in *R*. Measurement was done in buffer solution having a pH of 7. [7]